

Anticardiolipin antibodies in patients from Malaysia with systemic lupus erythematosus

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Abstract

Systemic lupus erythematosus (SLE) is highly prevalent in Malaysia, which has a mixed population of Malays, Chinese, and Indians. A quantitative enzyme linked immunosorbent assay (ELISA) was used to determine anticardiolipin antibody (aCL) levels (total immunoglobulin, IgG, and IgM) in 200 patients with SLE (164 Chinese, 26 Malay, and 10 Indian) attending the University Hospital of Kuala Lumpur, Malaysia, and 103 matched controls. Only 33 (16.5%) of the patients had raised aCL levels; 26 had raised IgG aCL, five IgM aCL, and two both IgG and IgM aCL. There was a low prevalence of raised levels of aCL in the population studied, which was seen in conjunction with a rare occurrence of thrombosis. The classical association of high aCL levels with thrombocytopenia and recurrent abortions was noted, though not with cerebral disease. The low prevalence of aCL in this study population of mixed racial origin contrasts with findings in European patients with SLE and lends support to the influence of local factors, be they genetic or environmental, on the clinical manifestations of this disease.

Antibodies to cardiolipin (aCL) are part of a family of autoantibodies directed against cell membrane phospholipids. These antibodies have assumed importance in the past decade because their presence in some patients, especially those with systemic lupus erythematosus (SLE), has been associated with venous and arterial thrombosis, cerebral disease, recurrent fetal loss, and thrombocytopenia.¹⁻⁴

In Malaysia SLE is common and found more often among the Chinese than the Indian or indigenous Malay populations.^{5, 6} Thus ethnic factors seem to have an influence on the susceptibility to the disease.

We wondered whether levels of aCL are affected in patients with lupus from Malaysia by the ethnic or environmental background or reflect the clinical manifestations of the disease. Using a standardised enzyme linked immunosorbent assay (ELISA),^{7, 8} we measured aCL levels in the sera of 200 ethnically distinct Malaysian patients with SLE attending a lupus outpatient clinic in Kuala Lumpur. Results were related to ethnic origins and clinical manifestations.

Subjects and methods

SUBJECTS

Serum samples were collected from 200 patients

examined at the SLE outpatient clinic, University Hospital, Kuala Lumpur, Malaysia, by three of the authors (FW, KV, and RI). All patients fulfilled the revised American Rheumatism Association criteria for the classification of SLE.⁹ Of the 200 patients studied, 114 were assessed clinically at the time of serum collection and demographic details and prevailing clinical manifestations were recorded. The overall group included 176 women and 24 men, ranging in age from 12 to 66 years (mean age 31.8) and comprised 164 Chinese, 26 Malay, and 10 Indian.

One hundred and three healthy volunteers (93 female, 10 male, mean age 31.9 years; range 17-56; 74 were Chinese, 17 Malay, and 12 Indian) acted as controls. Coded serum samples from all subjects were stored at -70°C until tested.

MEASUREMENT OF aCL

Measurements were carried out using a quantitative solid phase ELISA.⁷ Patient and control serum samples were first screened for aCL of all immunoglobulin isotypes with a peroxidase conjugated anti-IgG, IgA, and IgM antiserum (Sigma, Poole, Dorset) to detect antibodies bound to solid phase cardiolipin (Sigma). Serum samples were then tested for aCL of the specific IgG and IgM isotypes with peroxidase labelled anti-IgG and IgM antisera (Sigma). The results were expressed in the units recommended by the aCL standardisation international workshop.⁸ A strongly positive sample was calibrated against affinity purified IgG aCL and IgM aCL standards and assigned values of 200 G phospholipid (GPL) and 150 M phospholipid (MPL) units respectively. Total immunoglobulin aCL values were assigned arbitrary units using the GPL standard. Standard curves were prepared in each plate together with four known positive reference sera. Positive results were also graded as 'high', 'moderate', and 'low' as recommended in the above mentioned international workshop.⁸

STATISTICAL ANALYSIS

As the values of aCL followed a non-parametric distribution results were expressed as median and ranges and were compared by the use of the Wilcoxon's rank sum test. Correlations between age, disease duration, and aCL values were analysed by Spearman's rank regression analysis.

Statistical computations were performed using the statistical package for the social sciences (SPSS) on the University of London Computer Centre's Amdahl 5980/300.

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Table 1 Clinical manifestations in 114 patients from Malaysia with lupus. Results are given as number (%) of patients

Manifestation	Chinese (n=94)	Malay (n=15)	Indian (n=5)	Total (n=114)
Cutaneous	90 (96)	15 (100)	5 (100)	110 (96)
Articular	88 (94)	15 (100)	5 (100)	108 (95)
Renal	65 (69)	12 (80)	4 (80)	81 (71)
Haematological	48 (51)	6 (40)	1 (20)	55 (48)
Central nervous system	36 (38)	4 (27)	2 (40)	42 (37)
Pulmonary	33 (35)	4 (27)	3 (60)	40 (35)
Cardiac	28 (30)	4 (27)	1 (20)	33 (29)
Gastrointestinal	24 (26)	3 (20)	2 (40)	29 (25)
Sepsis	24 (26)	3 (20)	1 (20)	28 (25)
Thrombosis	0 (0)	1 (7)	0 (0)	1 (1)

Results

Full clinical details were obtained in 114 patients and are summarised in table 1. Articular, cutaneous, and renal manifestations were seen in most patients, being found in 95%, 96%, and 71% respectively; no differences in the prevalence of the manifestations between the racial groups were noted. Only one patient had a history of thrombosis (deep vein thrombosis).

Levels of aCL were considered positive when they exceeded the highest control value, which was 12.0 units for total immunoglobulin aCL, 7.5 GPL units for IgG aCL, and 5.0 MPL units for IgM aCL. Raised levels of aCL were found in 33/200 (16.5%) of the patients with lupus. Twenty six (13%) were IgG aCL positive, five (2.5%) were IgM aCL positive while two (1.0%) were positive for both isotypes. Values of total immunoglobulin and IgG aCL were significantly higher in patients than in controls (table 2), but similar between the ethnic groups. No 'high' positive levels of aCL were noted, but 9/28 patients had 'moderate' levels of IgG aCL and 4/7 had 'moderate' levels of IgM aCL.

Total immunoglobulin aCL values were significantly correlated with age both in patients ($R=0.19$, $p<0.05$) and controls ($R=0.19$, $p<0.05$). Of the aCL isotypes, only IgG aCL levels correlated significantly with age, and only in the patient group ($R=0.14$, $p<0.05$).

An inverse correlation was seen between total aCL levels and platelet count (table 3).

No differences were found in the prevalences of raised aCL between the three ethnic groups and no association was noted between aCL

levels and specific organ disease, including renal and cerebral disease. The patient with a history of thrombosis did not have raised levels of aCL at the time of testing. Five women had a history of more than one abortion and two of these had the highest IgG aCL levels detected (50 and 31 GPL units). In addition, four of them had thrombocytopenia, though no thrombotic episodes.

Discussion

This study shows a singularly low prevalence of anticardiolipin antibody and the virtual absence of thrombosis in a large series of patients with lupus living in Malaysia. It outlines other clinical peculiarities of SLE in Malaysia and confirms the association of aCL with thrombocytopenia but not with cerebral disease.

In this study no differences in disease manifestations among patients belonging to different racial groups were noted. Of interest, however, is the observation that renal disease was present in over 70% of cases, a prevalence much higher than those reported in other series, which ranged from 19.6 to 50.0%.⁹⁻¹⁰ This finding confirms a previous observation by Wang *et al*,¹¹ who noted that patients with lupus in Malaysia commonly have renal disease and a more severe form of membranous lupus nephritis than Western patients. In contrast with the prevalence of renal disease, thrombosis was extremely rare in our patients. This might be due to a bias in referral: thus patients with lupus presenting with thrombotic episodes might have been preferentially referred to non-SLE clinics. The clinical history of even our longstanding patients was consistently negative for thrombotic episodes, however, indicating that the low prevalence of thrombosis in our patients was genuinely an uncommon phenomenon.

It has been suggested that thrombotic manifestations in SLE are provoked by increased levels of antiphospholipid antibodies, especially aCL and 'lupus anticoagulant' antibody.² Of importance, therefore, is the observation that our patients had raised levels of aCL in their sera only occasionally, the antibody being present above normal in 16.5% of cases. This contrasts with findings in European patients with SLE, in whom the prevalence of aCL has been reported to vary from 39 to over 60%.¹²⁻¹⁵ The reason for this discrepancy is not known. Our patients had similar results despite their distinct ethnic origins, indicating that protection against thrombosis must be afforded by either genetic or environmental factors common to all three populations. Because our patients differ genetically we think that local factors, such as diet, are more likely to provide an explanation. High lipid intake is a known risk factor for thrombosis¹⁶ and may influence the production of aCL. If this hypothesis is correct, a low prevalence of raised aCL levels and thrombosis might be expected in patients with lupus living in South America and Asia, where the diet has a low fat content.¹⁶ Some support for this view comes from the studies by Saluja *et al* in India¹⁷ and by Chahade *et al* in Brazil.¹⁸

Table 2 Median and ranges of anticardiolipin antibody (aCL) values (units) in patients and controls

	Total immunoglobulin aCL (arbitrary units)	IgG aCL (GPL)
Controls	4.0 (1-12.0)	2.2 (0.3-7.5)
Patients	7.0 (2-131.0) $p<0.001$	4.0 (0.1-52.0) $p<0.001$

Table 3 Correlation of peripheral blood platelet numbers with anticardiolipin antibody (aCL) values

	Total immunoglobulin aCL	IgG aCL	IgM aCL
Controls			
R	0.012	0.064	0.086
P	0.451	0.264	0.199
Patients			
R	-0.193	-0.142	-0.086
P	0.024	0.074	0.107

R=Spearman's rank correlation coefficient.

The first study showed that aCL was present in only 27% of Indian lupus patients, in whom thrombosis was also rare, and the second study found aCL in just 20% of cases in Brazil. Two further reports from South Africa show that although clinical characteristics of SLE in the black population do not differ from those described in series from other parts of the world, the prevalence of aCL is low.^{19 20} These findings further indicate that regional determinants, be they environmental or genetic, have an important role in governing aCL production.

In our series we did not find the reported correlation between aCL and cerebral disease,²¹ possibly because this complication results from levels of aCL higher than those found in our study. On the other hand, we confirmed that levels of aCL correlate positively with age²²⁻²⁴ and negatively with the platelet count.^{3 25} The link between recurrent abortion and aCL³ was also supported by our findings as women with recurrent abortions had the highest levels of aCL.

Support for the notion that aCL causes disease is provided by two facts illustrated in this study. Firstly, the uniquely low occurrence of thrombosis in our patients with lupus was associated with the lowest prevalence of aCL yet reported in the disease. Secondly, the highest aCL levels were found in patients with thrombocytopenia. Moreover, it is becoming clear that autoantibodies are of two major immunoglobulin isotypes: IgG and IgM.²⁶ Whereas IgM autoantibodies have low affinity, broad reactivity, and low damaging capacity, IgG autoantibodies have high affinity and specificity for their antigen and high damaging potential.²⁶ In view of this the finding that aCL belongs predominantly to the IgG isotype may also be of pathogenic relevance.

In summary, the unexpected findings of absence of thrombosis and rarity of anticardiolipin antibodies in patients from Malaysia with lupus reinforce the association of the two and suggest an influence of regional factors on the manifestations of SLE.

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